

B-cell-attracting chemokine CXCL13 as a marker of disease activity and renal involvement in systemic lupus erythematosus (SLE)

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Abstract

Objectives. The chemokine CXCL13, also known as BCA-1 (B-cell-attracting chemokine-1) or BLC (B-lymphocyte chemoattractant), is a major regulator of B-cell trafficking. We have recently shown that excessive expression of dendritic cell-derived CXCL13 is a distinctive early event for nephritis in a murine model of systemic lupus erythematosus (SLE). Furthermore, in kidney biopsies from SLE patients, CXCL13 protein and mRNA are strongly expressed in B-cell-containing inflammatory lesions. Here, we ask whether serum levels of CXCL13 correlate with disease activity and renal involvement in SLE patients.

Methods. CXCL13 was measured in sera obtained from 91 patients with SLE and 40 healthy controls by ELISA methodology. Disease activity was calculated according to the SLE Disease Activity Index (SLEDAI).

Results. Median (IQR) serum CXCL13 concentrations were increasingly higher across the following groups: healthy controls [31.6 (26.8–41.3) pg/ml], SLE patients with inactive disease (SLEDAI <6) [68.2 (27.8–133.0) pg/ml, $P = 0.0006$ versus controls] and active disease [196.0 (75.9–416.8) pg/ml, $P = 0.0001$ versus controls] (inactive versus active $P < 0.0001$). Concentrations of circulating CXCL13 correlated with SLEDAI ($r = 0.56$, $P < 0.0001$) and double-stranded DNA titres ($r = 0.36$, $P < 0.0005$). Moreover, median CXCL13 concentrations were higher in patients with renal involvement [175.5 (105.3–422.6) pg/ml] compared to those without renal involvement [82.1 (42.9–219.8) pg/ml].

Conclusions. Our data indicate that increased level of CXCL13 is a feature of SLE that correlates with disease activity. Furthermore, CXCL13 might be a readily available surrogate marker to monitor the extent of aberrant B-cell (dys-)function.

Keywords: B cells; biomarker; chemokines; CXCL13; lupus nephritis

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease affecting different organ systems [1]. Chemokines have been shown to orchestrate migration and preferential sequestration of B and T cells in inflammatory lesions. The significance of chemokines in the pathogenesis of SLE and lupus nephritis (LN) is widely accepted [2,3] and recent interventional studies could demonstrate the therapeutic benefit of pharmacologic chemokine and chemokine receptor blockade in experimental SLE [4,5].

The chemokine CXC ligand 13 protein (CXCL13), also known as B-cell-attracting chemokine-1 or B-lymphocyte chemoattractant, is a CXC subtype member of the chemokine superfamily [6]. CXCL13 is one of the most potent B-cell chemoattractants and is constitutively expressed in the B-cell follicles of secondary lymphoid organs, pleural and peritoneal cavities, and in ectopic lymphoid follicles within the synovial membrane of patients with rheumatoid arthritis. Via its receptor CXCR5, expressed on follicular dendritic cells, CXCL13 is crucial for germinal centre formation [7–9]. Ishikawa *et al.* demonstrated that CXCL13 was markedly increased in the aged kidneys of NZB/W-F1 mice, a well-characterized mouse model for SLE, whereas it was barely detectable in the kidneys of young mice of the same strain [10]. These results could be confirmed by our previous studies in which we detected increased CXCL13 levels in untreated aged NZB/W-F1 mice, which could be reduced by treatment with CTLA4Ig and cyclophosphamide [11]. The role of CXCL13 is particularly interesting in the course of LN, since aberrant CXCL13 expression is sufficient to induce the formation of ectopic lymphoid tissues in non-lymphoid organs and thus could be responsible for the accumulation of inflammatory cells in the kidneys in SLE [10,12,13]. Furthermore, it is believed that the presence of ectopic lymphoid tissues promotes the local activation of T and B cells leading to exacerbation of disease [14]. Recently, we characterized the expression

Table 1. Characteristics of patients and controls

	Healthy controls	Patients total	Active SLE	Inactive SLE
No. of subjects	40	91	53	38
Females/males	26/14	80/11	49/4	31/7
Age (years)	47 (37–65)	44 (32–60)	39 (28–54)	47 (35–63)
SLEDAI score	–	7 (2–12)	11 (8–18)	2 (1–2)
dsDNA Ab (U/l)	–	5 (2–42)	21 (3–87)	2 (2–4)
C3 (g/dl)	–	1.30 (0.24–1.64)	1.28 (0.85–1.62)	1.39 (1.08–1.64)

SLEDAI = systemic lupus erythematosus disease activity index; dsDNA = double-stranded DNA.

profiles of 61 inflammatory molecules in the kidneys of NZB/W-F1 mice at different stages of LN [15]. We found that CXCL13 was one of only a few inflammatory markers that were expressed in the kidney at an early point of disease, suggesting a possible pathogenic role for disease manifestation.

Since kidney biopsies are still the gold standard for diagnosis of LN but bear potential complications, non-invasive tests to investigate kidney involvement are of great interest. Here, we ask whether serum levels of CXCL13 (1) are elevated in patients with active SLE, (2) correlate with disease activity and (3) indicate renal involvement in SLE patients.

Subjects and methods

Patients and controls

Ninety-one German Caucasian patients were consecutively recruited from the Departments of Rheumatology and Nephrology at Hannover Medical School (Hannover, Germany) and from the Medical Policlinic, University of Munich (Munich, Germany). No preselection of patients was performed. The study was done in accordance with the declaration of Helsinki and approved by the institutional review boards. Written informed consent was obtained from all participants. Disease activity was calculated according to the SLE Disease Activity Index (SLEDAI) [16]. For statistical comparison, the patients were classified as inactive (SLEDAI < 6) and active SLE (SLEDAI ≥ 6). The patients fulfilled at least four of the American College of Rheumatology Criteria for the classification of SLE [17,18]. The patients with acute infections and malignant disease were excluded from the study. Of 31 patients with renal involvement, LN class II was present in 7, class III in 6, class IV in 12 and class V in 1 patient according to the revised ISN/RPA classification [19]. A biopsy was not performed in five patients. The patients' characteristics are shown in Table 1.

Laboratory testing and quantification of CXCL13

Antibodies against dsDNA were analysed by radioimmunoassay (IBL, Hamburg, Germany). Complement C3 was quantified by a nephelometric method (Siemens, Marburg, Germany). Peripheral blood samples for quantification of circulating CXCL13 levels were immediately placed on ice, centrifuged at 1000 g for 10 min and stored at –80°C. Serum concentrations of CXCL13 were quantified by commercially available Quantikine ELISA methodology according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA, Catalog Number DCX130). All samples were measured in duplicates. Colour intensity was measured by a standard ELISA Reader (Tecan spectra mini, Crailsheim, Germany). Colour intensity correlates with the amounts of bound CXCL13. Quantikine kit standards were used for construction of standard curves.

Statistical analysis

The differences between patient groups and/or healthy controls were evaluated using the non-parametric Mann–Whitney test. The correlations between CXCL13 concentrations and parameters of disease activity were calculated with Spearman's test. Receiver operator characteristic (ROC) procedures identified optimal cut-off values for CXCL13 to differentiate between patients with and without LN. The statistical significance for all procedures was accepted at $P < 0.05$ probability levels (two-sided). Data

were displayed as median ± inter quartile ranges (IQR) unless otherwise stated. All statistical analyses were performed using the GraphPad Prism software (GraphPad Prism Software Inc. San Diego, CA, USA).

Results

CXCL13 concentrations are increased in patients with SLE

The median serum concentration of CXCL-13 was significantly higher in SLE patients [124.7 (49.9–303.0) pg/ml, $n = 91$] compared to healthy controls [31.6 (26.8–41.3) pg/ml, $n = 40$] ($P < 0.0001$). The CXCL13 levels in active SLE patients (SLEDAI < 6) were 3-fold higher compared to those in inactive SLE patients (SLEDAI ≥ 6) [196.0 (75.9–416.8) pg/ml, $n = 53$] [68.2 (27.8–133.0) pg/ml, $n = 38$; $P < 0.0001$]. Of note, the patients with inactive SLE still had higher CXCL13 levels than healthy controls ($P = 0.0006$) (Figure 1A).

CXCL13 correlates with parameters of disease activity

A positive correlation was observed between circulating CXCL13 levels and SLEDAI ($r = 0.56$, $P < 0.0001$) (Figure 1B). Likewise, CXCL13 concentrations showed a significant positive correlation with IgG double-stranded DNA (dsDNA) antibody titres ($r = 0.36$, $P < 0.0005$) (Figure 1C), whereas no correlation was seen with complement factor C3 ($r = -0.11$, $P = 0.33$) (Figure 1D).

CXCL13 is elevated in patients with renal involvement

Next we wanted to address the role of LN in circulating CXCL13 levels. Accordingly, median CXCL13 concentrations were higher in patients with renal involvement [175.5 (105.3–422.6) pg/ml] compared to patients without renal involvement [82.1 (42.9–219.8) pg/ml] (Figure 2A). Finally, we estimated the potential use of CXCL13 as a marker for renal involvement by ROC curve procedures. The AUC was 0.69 ± 0.058 (95% CI 0.57–0.8; $P = 0.004$). A calculated CXCL13 cut-off value of >162.2 pg/ml resulted in 72% specificity and a sensitivity of 61% in discriminating renal from non-renal involvement in SLE. CXCL13 levels were not different across different LN classes ($P = 0.264$; Kruskal–Wallis test). However, CXCL13 was outperformed by the discriminatory ability of anti-dsDNA antibody titres [AUC 0.76 ± 0.05 (95% CI 0.66–0.86) $P < 0.0001$] when an optimal cut-off titre of >9 was used (75% specificity and 74% sensitivity). The anti-dsDNA antibody titres were higher in patients with renal involvement [24 (7–87) U/l] compared to patients without renal involvement [2 (2–13.5)

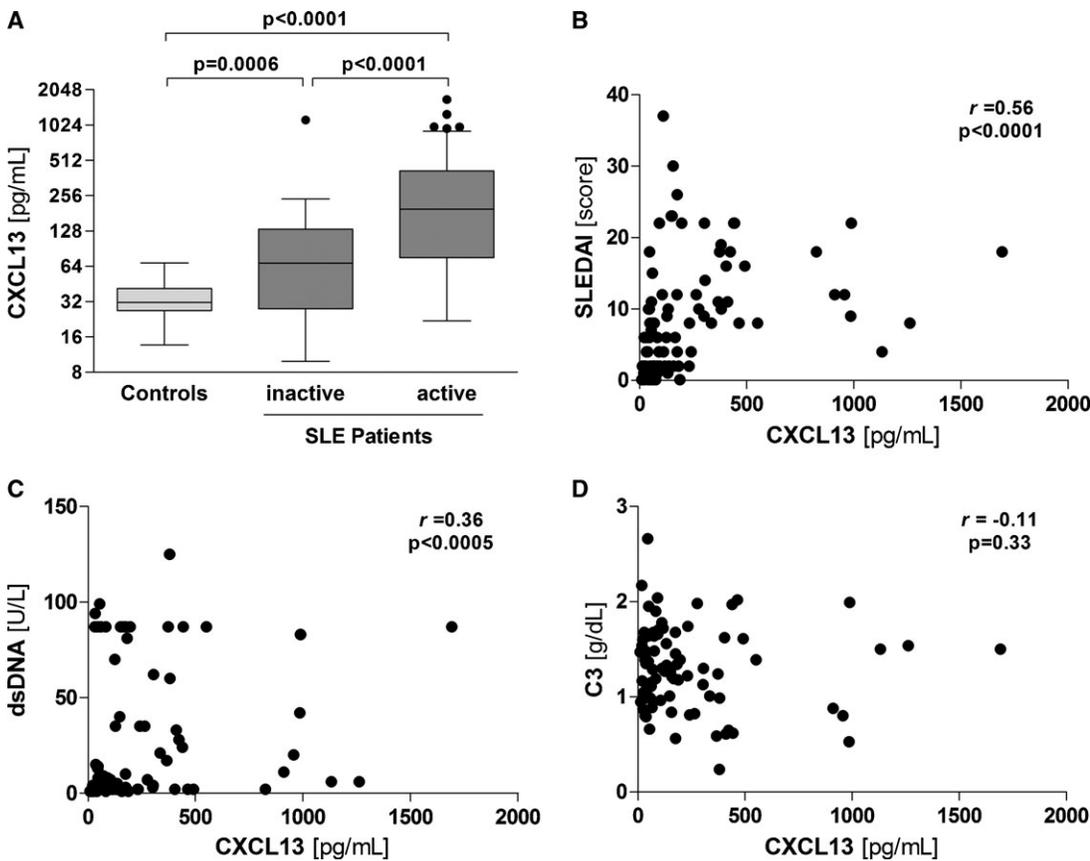


Fig. 1. Circulating CXCL13 concentrations are increased in active SLE and correlate with disease activity. (A) Box-and-whisker plots showing serum concentrations of CXCL13 in 91 patients with SLE and 40 healthy controls. SLE patients were classified according to inactive (SLEDAI <6; $n = 38$) or active (SLEDAI ≥ 6 ; $n = 53$) disease. Horizontal bars indicate median values, whiskers indicate 1.5 times the interquartile distance and dots indicate outliers. Scatter plots showing the correlation of circulating CXCL13 with (B) disease activity (SLEDAI score), (C) double-stranded DNA antibody titre and (D) complement C3 levels (Spearman's test).

U/l] (Figure 2B). The C3 level was not able to discriminate between patients with and without LN [AUC 0.55 ± 0.07 (95% CI 0.41–0.68) $P = 0.49$].

Discussion

B-cell lymphocytes play an important role in the pathogenicity of SLE [20]. CXCL13 is the only chemokine so far which is known to specifically chemoattract B cells through the interaction with its receptor CXCR5. In the present cross-sectional clinical investigation SLE patients are characterized by (1) excess serum levels of circulating CXCL13; (2) serum concentrations of circulating CXCL13 correlate with disease activity (SLEDAI) and B-cell activation (ds-DNA titres); and (3) median CXCL13 concentrations are higher in patients with renal involvement.

In experimental SLE, several lines of evidence suggest an important role of the B-cell-attracting chemokine CXCL13 in aberrant/defective B-cell trafficking. It has been shown that CXCL13 expression is sufficient to induce the formation of ectopic lymphoid tissues in non-lymphoid organs, leading to exacerbation of disease via accumulation of inflammatory cells in the kidneys in SLE [10,12,13]. Interestingly, the renal expression of CXCL13 markedly increases with age, but is barely detectable in young SLE-prone mice

[10]. In a recent study, we identified activated macrophages and dendritic cells as the main source of CXCL13, whereas CXCR5 was predominantly expressed by B cells in the kidneys of nephritic SLE mice. Moreover, the renal expression of CXCL13 in diseased NZB/W-F1 mice could be effectively reduced by treatment with CTLA4Ig and cyclophosphamide [11].

In SLE patients, circulating CXCL13 has never been assessed as a marker of disease activity, although there is extensive rationale in the literature to suggest that CXCL13 contributes to the pathogenesis of SLE and LN. Circulating CXCL13 has recently been proposed as a potential marker of disease activity in patients with rheumatoid arthritis [21], and excess levels of circulating CXCL13 correlate with active cutaneous vasculitis in Hepatitis C virus-related cryoglobulinaemia [22]. Well in line, serum concentrations of circulating CXCL13 correlated with disease activity (SLEDAI) and B-cell activation (ds-DNA titres) in the present study. Of note, CXCL13 serum levels remained significantly elevated in SLE patients with inactive disease (SLEDAI <36) compared to healthy controls, probably indicating aberrant B-cell trafficking even in remission.

Intrarenal B-cell aggregates are described in different forms of inflammatory kidney disease, including LN, and correlate with a worse clinical outcome [23–25]. We could

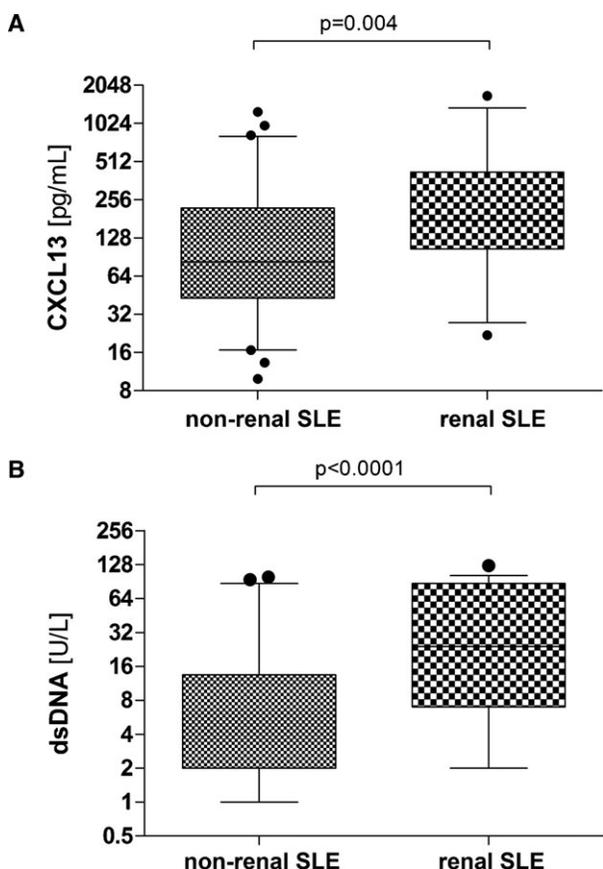


Fig. 2. Circulating CXCL13 concentrations are elevated in lupus nephritis. Box-and-whisker plots showing serum concentrations of (A) circulating CXCL13, and (B) double-stranded DNA antibody titres in 31 SLE patients with biopsy-confirmed lupus nephritis and 60 SLE patients without lupus nephritis.

previously identify CXCL13 as a key chemokine during disease manifestation and progression of LN in NZB/W-F1 mice. Intriguingly, CXCL13 concentrations were higher in patients with LN in the present study. It is tempting to speculate that high CXCL13 serum levels may be a consequence of high local expression in the kidneys. However, peripheral dendritic cells could be an alternative source of circulating CXCL13. Since our study is a cross-sectional observational study, these speculations would deserve further investigation.

In conclusion, we could show that an increased level of CXCL13 is a distinctive feature in SLE, even in patients with low disease activity. According to our previous report in experimental SLE [11], CXCL13 might be a readily available surrogate marker to monitor the extent of aberrant B-cell (dys)function.

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Conflict of interest statement. None declared.

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The clinical relevance of a repeat biopsy in lupus nephritis flares

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Abstract

Background. The clinical utility of performing repeat biopsies during lupus nephritis flares is questionable and data pointing towards frequent class switches are based on the old WHO classification. This retrospective study investigates the hypothesis that clinically relevant switches from proliferative to non-proliferative lesions and vice versa as determined by the new ISN/RPS classification are a rare event and that repeat biopsies are unnecessary in many cases.

Methods. Thirty-five patients with lupus nephritis and one or more repeat renal biopsies were included. Eighty-four biopsies were blindly reassessed according to the ISN/RPS classification.

Results. Twenty-five patients had one repeat biopsy, 6 patients had two and 4 patients had three repeat biopsies. Forty-nine comparisons between reference and repeat biopsies could be made. In 25 cases (54.3%), there was no shift in ISN/RPS class on repeat biopsies. In 41 instances, paired biopsies showed proliferative lesions both on reference and repeat biopsies, whereas five of six cases with non-proliferative lesions on a reference biopsy switched to proliferative lesions on a repeat biopsy. Clinically significant class switches during lupus nephritis flares were more frequent in patients with non-proliferative lesions in their reference biopsy ($P < 0.001$).

Conclusion. The results show that patients with proliferative lesions in the original biopsy rarely switch to a pure non-proliferative nephritis during a flare. Therefore, a repeat biopsy during a lupus nephritis flare is frequently not necessary if proliferative lesions were found in the refer-

ence biopsy. However, in the case of a non-proliferative lesion in the reference biopsy, class switches are frequently found and repeat biopsies are advisable.

Keywords: ISN/RPS classification; kidney biopsy; lupus nephritis; proliferative lesions; SLE

Introduction

A renal biopsy is a pivotal step in determining the nature of renal involvement in patients with lupus nephritis. Up to 60% of patients with systemic lupus erythematosus (SLE) develop lupus nephritis [1]. Six classes of lupus nephritis are distinguished in the current classification of the International Society of Nephrology and the Renal Pathology Society (ISN/RPS). Classification and treatment decisions strongly depend on the findings in the renal biopsy. The diagnosis of lupus nephritis cannot be based on clinical features alone (e.g. proteinuria, rising serum creatinine, active sediment), since the clinical features do not permit a reliable prediction of the type of SLE nephritis [2,3]. Kidney diseases due to other causes than lupus nephritis may also need to be excluded as a cause of renal damage [1].

Relapses occur frequently in patients with lupus nephritis, even after an initial complete remission [4]. To determine the most effective treatment in the case of a lupus nephritis flare, a number of authors advise to perform repeat biopsies [1,5–8]. Based on such findings, it has been hospital policy at the Leiden University Medical Centre (LUMC)



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